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**Original Research Article** 

DOES PRESTERILIZATION HAS ANY INFLUENCE ON CONSERVATIVE AND ENDODONTIC MATERIAL STERILITY, IMMEDIATELY AFTER UNPACKING AND POST EXPOSURE TO CLINICAL ENVIRONMENT FOR 15 DAYS - AN EX VIVO STUDY.

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## **Abstract**

**Background:** Instruments used in the operative area are colonised by various microbial organisms during usage. Sterilization is an important prerequisite to avoid cross contamination.

**Purpose of the study:** The purpose of this study was to evaluate whether presterilization has any influence on conservative and endodontic material sterility, immediately after unpacking and post exposure to clinical environment for 15 days.

**Methods:** Gutta percha cones, Rubber dam clamps, Impression trays, Dental burs (#245 bur, Access opening bur) were tested. Materials were randomly sampled at 2 time points (t0, at package opening; t1, at 15days) during their clinical usage. Vortexing, centrifuging, streak and inoculation was done. Colony formation under aerobic conditions was observed on media before and after presterilization.

**Statistical analysis:** Wilcoxon matched pairs test was done for comparison of data before and after pre sterilization with status of aerobic organisms at to (immediately after opening) and t1 (during clinical usage).

**Conclusion:** Presterilization effectively eliminates cross contamination of various aerobic microorganisms in conservative and endodontic material immediately unpacked and post exposure to clinical environment for 15 days.

## Introduction

Infection control and modes of sterilization are the key factors to avoid cross transmission of infection in the field of dentistry. Transmission of disease or infection is noted with improper sterilization of reused instruments.<sup>1</sup>

Dental burs are used in clinical dentistry for various procedures some of which includes caries excavation and access cavity preparation. During these procedures burs may become heavily contaminated with necrotic tissue, saliva, blood and potential pathogens and identified as potential vehicle for cross infection. The most commonly used methods of sterilization includes soaking of burs in commercially available disinfectors followed by manual cleaning or, using ultrasonic bath or, autoclaving.<sup>2</sup>

Although most gutta-percha cones appear to be sterile, there is a risk of contamination from both air-borne and physical sources during their storage. In contrast to the care that is taken during cleansing the canals, obturation is often accomplished using gutta-percha cones directly

from storage containers without regard to their sterility.<sup>3</sup>

Dental impressions can give rise to the transmission of microorganisms and infections. Rubber dam clamps should be cleaned and sterilized immediately after the procedures to prevent the cross contamination and to increase the life of clamp.<sup>3</sup>

In this study, culture techniques have been used for the identification of bacterial contamination. The aim of this study was to evaluate whether presterilization has any influence on conservative and endodontic material sterility, immediately after unpacking and post exposure to clinical environment for 15 days.

#### **MATERIALS AND METHODOLOGY**

The present study was conducted in Department of conservative dentistry and endodontics, Mamata dental college, Khammam.

✓ Samples examined include five samples each of Gutta percha cones, Rubber dam clamps, Impression trays, Dental burs(#245 bur, Access opening bur) at two specific intervels of time i.e,

-Samples immediately after opening the original packaging (t0) under aseptic conditions, before and after presterilization. (Figure 1)

-Samples during clinical storage and usage (t1) after using for 15days, before and after presterilization.

Samples tested before presterilization at both the intervals of time were placed in Phosphate-buffered saline(PBS) & incubated for 1hr.Plain PBS was used as negative control (Figure 2).All the samples were Vortexed (Figure 3) and centrifuged for 15mins at 4200rpm(Figure 4).The supernatant was poured off and 50µl of sediment was inoculated on to each Blood Agar, MacConkeys Agar and Sabourad's Dextrose Agar(SDA) and streaked (Figure 5).Inoculated plates were incubated at 37°for 48 hrs aerobically and observed for colony formation.

Presterilisation was done for rubber dam clamps and impression trays in autoclave at 121 C at 15 lbs for 15 min (Figure 6).2% gluteraldehyde was used for dental

burs and Gutta percha (Figures7a,7b). The above procedure was repeated to observe for colony formation. Inoculated plates with colony formation were counted (each set with atleast one colony is counted as one) (Figure 8, 9). Statistical analysis was done.

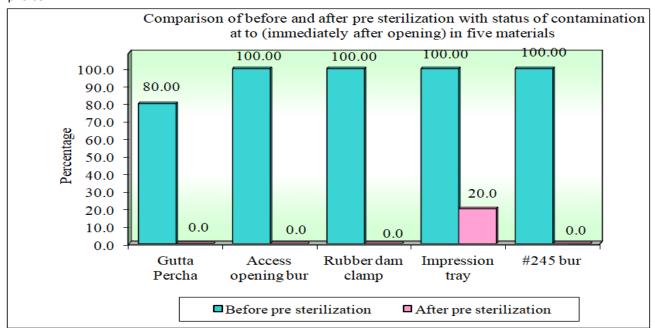
#### STATISTICAL ANALYSIS AND RESULTS:

Wilcoxon matched pairs test was done for comparison of data before and after pre sterilization with status of aerobic organisms at to (immediately after opening) and t1( during clinical usage) in all the five materials.80% of Gutta percha cones were found with the presence of aerobic microorganisms which became sterile after presterilization. All the samples from other groups were contaminated with aerobic microorganisms which were sterilized after presterilization except impression trays. At t1,all the samples were contaminated before presterilization. 80% of access opening burs and rubber dam clamps were sterilized after presterilization.

**Table 1**: Comparison of before and after pre sterilization with status of contamination at to (immediately after opening) in five materials by Wilcoxon matched pairs test.

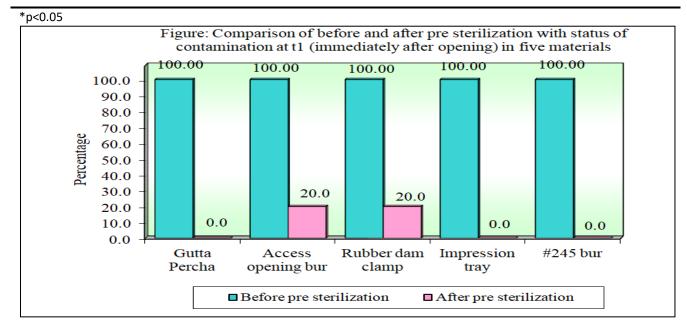
Materials	Before pre sterilization				After pre sterilization				Total	Z-value	p-value
	With contamination	%	Without contamination	%	With contamination	%	Without contamination	%	-		
Gutta Percha	4	80.0	1	20.0	0	0.0	5	100.0	5	1.8257	0.0679
Access opening bur	5	100.0	0	0.0	0	0.0	5	100.0	5	2.0226	0.0431*
Rubber dam clamp	5	100.0	0	0.0	0	0.0	5	100.0	5	2.0226	0.0431*
Impression tray	5	100.0	0	0.0	1	20.0	4	80.0	5	1.8257	0.0679
#245 bur	5	100.0	0	0.0	0	0.0	5	100.0	5	2.0226	0.0431*
Total	24	96.0	1	4.0	1	4.0	24	96.0	25	4.1973	0.0001*

<sup>\*</sup>p<0.05



**Table 2:** Comparison of before and after pre sterilization with status of contamination at t1 (immediately after opening) in five materials by Wilcoxon matched pairs test.

Materials	Before pre sterilization				After pre sterilization				Total	Z- value	p- value
	With contamination	%	Without contamination	%	With contamination	%	Without contamination	%	-	value	value
Gutta Percha	5	100.0	0	0.0	0	0.0	5	100.0	5	2.0226	0.0431*
Access opening bur	5	100.0	0	0.0	1	20.0	4	80.0	5	1.8257	0.0679
Rubber dam clamp	5	100.0	0	0.0	1	20.0	4	80.0	5	1.8257	0.0679
Impression tray	5	100.0	0	0.0	0	0.0	5	100.0	5	2.0226	0.0431*
#245 bur	5	100.0	0	0.0	0	0.0	5	100.0	5	2.0226	0.0431*
Total	25	100.0	0	0.0	2	8.0	23	92.0	25	4.1973	0.0001*



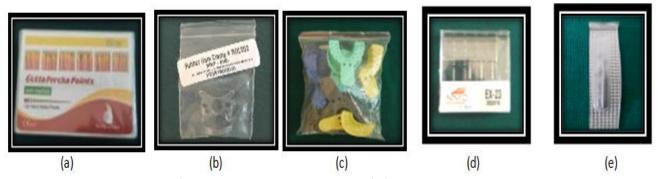


Figure 1: Samples immediately after opening the original packaging (t0).



Figure 2: Plain PBS as negative control





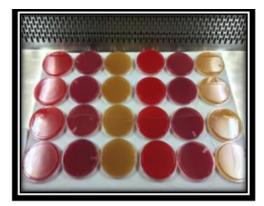
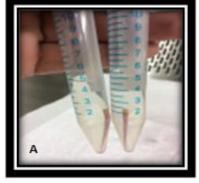


Figure 3: Vortex

Figure 4: Centrifuge

Figure 5: Blood Agar, MacConkeys Agar & Sabourads Dextrose Agar(SDA)





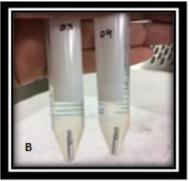


Figure 6: (Autoclave)

Figure 7a,b: Gutta percha and dental burs in 2% Gluteraldehyde at to and t1



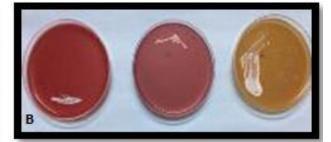


Figure 8 A,B: Gutta percha at to and t1 before presterilization

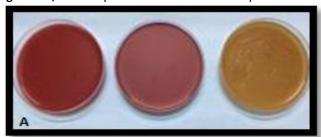




Figure 9 A,B: Gutta percha at to and t1 after presterilization

# Discussion

Oral cavity is inhabited by various aerobic and anaerobic bacteria and asepsis in conservative and endodontic therapy could be threatened with contaminated materials. Patient safety comes first of all. Many studies examine sterilization and disinfection of dental instrumentation and the safety of reusing them when they are exposed to the dental office environment or physically handled, to prevent cross contamination. <sup>2</sup>

Presterilization is "The repeated application of the terminal process designed to remove or destroy all viable forms of microbial life, including bacterial spores, to an acceptable sterility assurance level." This study determined the bacterial contamination of endodontic and conservative materials before and after clinical use and storage for 15 days in aerobic conditions when presterilised.

The results of the present study shows that presterilization of Gutta-percha cones before use is important. Although they are manufactured under aseptic conditions, most manufacturers do not claim that they are sterile. They also can be contaminated by handling, by aerosols and during the storage process. Studies done by Kos et al, Doolittle et al etc have proved that Gutta percha cones in unopened boxes are likely to be sterile.5 Authors like Linke and Chohayeb noted that environmentally exposed cones were no longer sterile and can be contaminated by various facultative anaerobes such as Staphylococcus, E.faecalis ,Aerobes such as Bacillus species and many anaerobes. Because gutta-percha cones are damaged by standard hightemperature sterilization methods, dentists resort to chemical methods to maintain the chain of asepsis. 1,6

In this study,2% gluteraldehyde has been used for decontamination for gutta-percha .Culture in aerobic conditions after presterilization with gluteraldehyde has shown absence of any growth of microorganisms at both intervals of time.This study is in accordance with the results obtained by various authors like Frank et al. 6

Sterilization procedures were successful for burs that had not been previously contaminated by organic debris and also after storage under clinical conditions. 2% Gluteraldehyde used in this study proved to be an effective way of eradicating aerobic organisms. The results are according to studies done by Nivashini et al which show reduction but not complete elimination of microorganisms post sterilization. Morrison et al who concluded that dental burs are not sterile when purchased and should be cleaned and sterilized before use.<sup>7</sup>

Rubber dam clamp and impression trays used in this study were presterilized according to manufacture instructions. After autoclaving, there was no growth observed under aerobic conditions during culture. Many reports stated that plastic impression trays are better discarded rather than reused, Steam sterilization is effective than dry heat sterilization.<sup>7</sup> All surfaces that

have been splashed or touched by human body fluids must be disinfected with a hospital-grade disinfectant that has been registered with the Environmental Protection. All the impression trays must be cleaned, disinfected and sterilized before using them for the first time and then after every use and every time they are contaminated.

#### Limitations

The present study proves the sterility of equipment in strict aerobic conditions and in limited duration of 15 days. Future studies are required to analyse the microorganisms present and various ways to eliminate them.

#### Conclusion

Within the limited conditions, it can be concluded that presterilization effectively eliminates cross contamination of various aerobic microorganisms in conservative and endodontic material immediately after unpacked and post exposure to clinical environment for 15 days.

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